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A N N U A L P R O G R E S S R E P O R T

Including Technical Report For January 1954

TO

OFFICE OF NAVAL RESEARCH
UNITED STATES DEPARTMENT OF THE NAVY

BIOLOGICAL SCIENCES DIVISION
CLINICAL BRANCH
LT. COMMANDER WILLIAM J. PERRY, HEAD

FROM

BURKE RESEARCH COMPANY

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"Glycerol Pectate as a Blood Plasma Replacement"

REPORT NO. 21

For Period: May 5, 1952 to January 31, 1954

URS F. NAGER

Glycerol Pectate as a Blood Plasma Replacement

Summary

Among the naturally occurring hydrophilic colloids, those of citrus peel have been investigated for use as a blood plasma replacement. According to earlier biological studies, degraded citrus pectin, a partial methyl ester of polygalacturonic acid (60 to 70% carboxyl groups methylated), has shown suitable oncotic properties and a low degree of toxicity. However, the so-called pectin sols have not gained the general acceptance of the medical profession because of certain shortcomings which may have been due to the high percentage of free carboxyl groups responsible for the precipitation of calcium pectinate.

Attempts have thus been made to prepare suitable derivatives of pectic acid (product used having between 10 to 20% of the carboxyl groups methylated) that would no longer precipitate with calcium ions. With the intention of blocking the free carboxyl groups of pectic acid, by esterification with a hydrophilic alcohol, propylene glycol pectate has been prepared. Biological studies on preparations esterified beyond the point where calcium precipitation was possible have shown best results with propylene glycol pectate samples containing about 15% residual unesterified carboxyl groups, but certain toxic symptoms attributable to a propylene glycol ester were encountered in these studies.

In comparative biological studies, glycerol pectate has proven to be an effective blood plasma replacement free of toxic side reactions. Its preparation has been accomplished by esterification of a commercial pectic acid with glycidol. In studies on artificially hemorrhaged dogs, effective restoration and maintenance of plasma volume and blood pressure have been obtained with 4% solutions of glycerol pectate in saline at a relative viscosity of around 4 and at an esterification degree of 85%. In vitro studies have demonstrated that the oncotic properties of glycerol pectate are primarily due to the unesterified carboxyl groups. Hydration and/or charge effects on these carboxyl groups appear to contribute to the molecular configuration; the number average molecular weight seems to be considerably below that of plasma.

Introduction

Ever since the last war there has been a tremendous effort placed on the development of a satisfactory artifical plasma replacement fluid for the treatment of hemorrhage, shock, burns and other conditions. Although adequate amounts of human blood plasma have been available for normal needs it became obvious that these supplies would never be sufficient to meet the requirements of the armed forces and the civilian population in case of a major catastrophe. In spite of numerous attempts only a few synthetic preparations are available today which approach the requirements of a satisfactory plasma replacement fluid, the two of most importance being dextran and polyvinyl pyrrolidone (PVP). Neither human plasma nor the just mentioned most promising synthetic plasma replacers have yet reached the stage where they could be used universally without any ill side effect.

Since relatively few compounds have so far been investigated as synthetic plasma replacers, very little is known with regard to the preferred class of compounds that will yield the most satisfactory material. However, present knowledge indicates that a polysaccharide-type compound may be superior to a protein-type or strictly synthetic compound. It is only logical to assume that besides dextran a variety of other materials exist in nature useful as plasma replacers. The objective of this project was, therefore, to search in the group of naturally occurring hydrophilic colloids for such a material. Of particular interest appeared to us the plant colloids among which notably those of citrus peel (pectin), cactus and plantago are available in unlimited supply. Due to time limitations placed on the extent of our research, we had to restrict our studies to one material. Pectin was selected since it already had shown distinct plasma replacement properties, was known to be non-toxic and did not accumulate in the body tissues. However, in the manner in which pectin had been heretofore prepared and used, pectin had never gained the general acceptance of the medical profession.

Earlier Studies on Pectin Sols

Pectin solutions introduced by Hartman et al[1], subsequently also referred to as pectin sols[2], have been prepared from commercial Pectin N.F. by thermal degradation of a 1.5% solution to a relative viscosity of about 4. Such prepared pectins represented a partial methyl ester of polygalacturonic acid with about 30-40% free carboxyl groups as sodium salts.

The affinity of the unesterified carboxyl groups of pectin towards calcium, other alkaline earth and heavy metal ions is well known. In fact, pectin is made use of, for example, in the jelly manufacturing as a thickening agent or in medicine as a detoxifying agent in heavy metal poisoning.

The percentage of free carboxyl groups of these pectin sols was in the range where precipitation with calcium ions had to be expected. Furthermore, it would be anticipated that the great number of free acid groups present in such a polymer produces a distinct cation- exchange behavior with selective affinity for cations according to their size and charge. For example, such a polymer may bind calcium more strongly than sodium. It appeared obvious that pectin sols when introduced as the sodium salt into the circulatory system would react, by double decomposition, with other salts such as those of multivalent ions; such reaction in turn may seriously affect the electrolyte balance. Recent animal studies made at the Henry Ford Hospital on salts of polygalacturonic acid, having about 25% of its carboxyl groups esterified with methanol, caused extensive sludging in the circulatory system followed by immediate death.

[1] F.W. Hartman et al., Ann. Surg. 114, 212 (1941); J.A.M.A. 118, 1161 (1942); 121, 1137 (1943)

[2] G.H. Joseph, Pectin Sols for Parenteral Use. Review publ. by California Fruit Growers Exchange, Ontario, California. (1950)

The original concept in the present program, was to block off the free carboxyl groups of pectin to prevent precipitation of calcium pectate or pectinate and to eliminate the chances of deposition of such salts in the tissue. The plan was to investigate completely esterified derivatives of pectin. Essentially two groups of highly esterified derivatives of polygalacturonic acid are known: (a) alkyl polygalacturonates, e.g., methyl polygalacturonate obtained by reacting polygalacturonic acid or its naturally occurring partial methyl ester (pectin) with diazomethane at low temperatures and (b) hydroxy alkyl pectates, e.g., hydroxyethyl polygalacturonate prepared by reacting polygalacturonic acid with ethylene oxide in the presence of water. Of these two groups comprising derivatives of the carboxyl groups the hydroxy alkyl esters were preferred because of solubility considerations; the ultimate aim was the preparation and testing of glycerol pectate (glycerol polygalacturonate). Glycerol pectate promised to be the derivative of choice because of the extremely low toxicity and the possibility of metabolism of glycerol if it should be liberated in the body by initial metabolism of the infused pectate ester. Since but little information was available on the preparation of glycerol pectate, and since glycidol, the esterifying agent required for its preparation, was difficult to make, it was first attempted to prepare the propylene glycol pectate to develop a practical preparative method and to verify our working hypothesis.

Studies on Propylene Glycol Pectate

Although original attempts were made to prepare our own polygalacturonic acid from citrus peel, for economical reasons resort was made to commercially available pectin preparations. Among these Pectic Acid No. 75[3] and Pectin N.F.[3] proved to be most satisfactory starting materials available in unlimited quantities. Pectic Acid No. 75 was found to have 80% free carboxyl groups, the rest being esterified by methyl groups. Following the general principle proposed by Deuel[4] for esterification with ethylene oxide, Pectic Acid No. 75 was esterified with propylene oxide in the presence of water. No matter what variations of the reaction conditions were made, a considerable excess of propylene oxide (at least 9 times the theoretical requirement) was needed as a substantical portion of propylene oxide was hydrolyzed to propylene glycol due to the presence of water. Studies with regard to the reaction mechanism revealed that a small amount of alkali was a desirable catalyst for esterification. This was already present in Pectic Acid No.75 but had to be added in esterification studies with purified pectic acid.

[3] Sunkist Growers, Ontario, California
[4] H. Deuel. Helv. Chim. Acta., 30, 1523 (1947)

It was then discovered that esterification may be accomplished more conveniently and economically in a heterogenous system, where Pectic Acid No. 75 was suspended either in an excess of propylene oxide or in an appropriate solvent thereby reducing the amount of water to a minimum. In this manner, complete esterification was achieved with as little as twice the stoichiometric requirement of propylene oxide; e.g., with a charge consisting of 10 parts Pectic Acid No. 75, 7.5 parts propylene oxide, 40 parts pentane, 1 part water complete esterification resulted with a reaction time of 20 hours at 35°C. At the end of the esterification, indicated by neutral reaction of the solids, the ester was isolated simply by collecting the solids by filtration, rinsing with acetone and drying. The crude propylene glycol pectate was then dissolved in water, bleached with chlorine dioxide and clarified. The filtrate diluted to proper concentration was then treated with two volumes of acetone to precipitate the ester. The precipitate was isolated by decanting the superatant liquid, slurrying the solids several times with fresh acetone and finally drying in vacuo.

Several batches of propylene glycol pectate so prepared were submitted to Dr. F. W. Hartman of Henry Ford Hospital Laboratories for evaluation on animals*. In loading experiments on mice and rabbits no signs of acute toxicity were found. When administered to dogs subjected to severe experimental hemorrhage (up to 85% blood withdrawn in 3 steps) the preparations positively were shown to be capable of restoring and maintaining plasma volume and blood pressure. The levels employed were in the range of 3.5 to 4.0% in saline adjusted to pH 7.3. However, when given to dogs in large doses, occasionally serious side reactions were observed such as edema of the lungs and the heart. The intensity of these toxic reactions were variable and appeared to be associated with particular batches of propylene glycol pectate. In vitro, differences in oncotic pressure could be demonstrated among the various products. A careful examination of all the samples submitted for testing revealed a variation in the degree of esterification. A high incident of toxic reactions and low oncotic pressure could be correlated with a high degree of esterification. A series of tests on dogs with preparations adjusted to various degrees of esterification revealed that highly esterified preparations were unable to support plasma volume and blood pressure, and that low esterified material, in turn, was excreted too rapidly. Furthermore, these studies clearly indicated an optimal degree of esterification of around 85%. Consequently, a certain amount of free carboxyl groups was essential to attain satisfactory oncotic pressure in vitro and adequate support of blood pressure in vivo. Nevertheless, propylene glycol pectate was not further pursued because of the toxic side reactions encountered which were very much alike those reported on polypropylene glycols and esters thereof.

*All references herein to such animal evaluation studies are based on oral reports only.

Studies on Glycerol Pectate[5]

Efforts were then devoted to the preparation of glycerol pectate from Pectic Acid No.75 and glycidol. After considerable initial difficulties glycidol was finally obtained in 60-70% yield by reacting glycerol alpha-monochlorohydrin with caustic soda. Due to scarcity of glycidol only a limited number of esterification studies could be made and attempts to esterify in a heterogenous system have been unsuccessful so far. However, these studies have shown that glycerol pectate can be prepared in a homogenous system using a charge of 1 part of Pectic Acid No.75, 2 parts of glycidol and 4.5 parts of water and a reaction time of 24 hours at 40°C. Glycerol pectate was then isolated according to the procedure outlined in Table I.

TABLE I

Preparation of Glycerol Pectate

Pectic Acid #75 (2.4 Kg) + Glycidol (4.8 Kg) + H_2O (10.9 Kg)

21%-$COOCH_3$
68%-COOH
11%-COONa

↓ Agitated 24 hours at 40°

Glycerol Pectate Reaction Mixture

↓
a) bleached with 4.5 liters ClO_2 solution (approx. 0.3%)
b) diluted with 5.5 liters H_2O
c) filtered on K-7 clarifying pad precoated with Dicalite SS

Filtrate

↓
a) precipitated with equal volume of acetone (about 22 liters)
b) isolated by decanting supernatant liquid, reslurrying precipitate with acetone, and drying

Crude Glycerol Pectate

↓
a) redissolved in 24 liters H_2O
b) added 180 ml saturated NaCl solution
c) precipitated with 24 liters acetone and isolated as above

Purified Glycerol Pectate (2.1 Kg)

Degree of esterification: 88%
$\eta_{rel.} \sim 7$ (c = 4%)

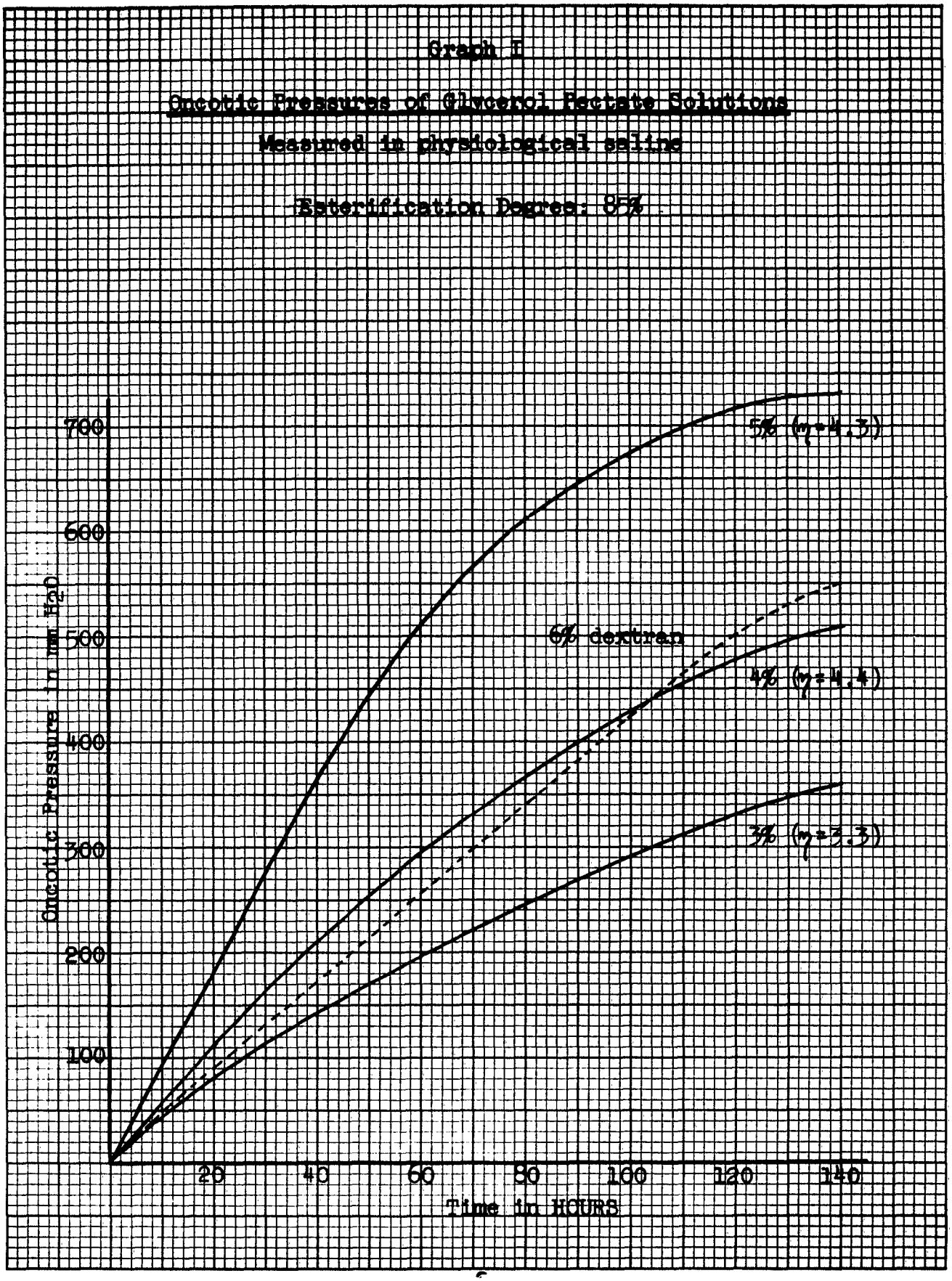
[5]U.S. Patents being applied for.

The purified glycerol pectate prepared as outlined above, dissolved much more rapidly and showed a greater water solubility than propylene glycol pectate. It gave practically colorless and non-foaming solutions. For reasons of comparison with the propylene glycol ester, glycerol pectate was tested on animals at a concentration level of 4% in the relative viscosity range of 4 to 5 and with a degree of esterification of 85%. Solutions suitable for intravenous administration conforming to the above specifications were prepared in the following manner. The purified glycerol pectate was dissolved in the proper amount of 0.9% saline to give a 4% solution and then clarified by filtration in the presence of a small amount of Celite, analytical grade. The filtered solution was thermally degraded at 100°C to lower the relative viscosity to around 4, which usually required about two hours. The degraded solution was checked for degree of esterification by titrating the free acidity of a deionized aliquot. This determination permitted the calculation of the amount of alkali required to adjust the solution, by partial saponification, to 85% esterification. For stability reasons, it was preferred to keep the degraded solution at the natural pH (of around 4) and add the alkali just prior to administration.

In studies at the Ford Hospital, glycerol pectate proved to be a very promising plasma replacer. According to Dr. Hartman's findings, glycerol pectate did not give the toxic side reactions observed with the propylene glycol derivative. Plasma volume of hemorrhaged dogs was quickly restored to normal and maintained, and no evidence of deposition in the tissues could be demonstrated.

In view of these results, Dr. W. J. Perry of the Office of Naval Research, arranged a more extensive evaluation of glycerol pectate at the Naval Medical Field Research Laboratories of Camp Lejeune. The original testing program proposed by Capt. C. B. Galloway and Dr. S. W. Handford, was to study glycerol pectate at concentration levels of 3, 4, 5 and 6% at a relative viscosity of around 4. Also, it was agreed to study the 4% level at a relative viscosity of around 3.

Solutions in saline containing 3,4,5 and 6% glycerol pectate were prepared as described above and then thermally degraded at 100°C. to a relative viscosity of around 4, requiring 1,4,7 and 11 hours, respectively. Due to the prolonged heat treatment, the 6% solution showed considerable darkening and was therefore considered unsatisfactory for use in animals. Oncotic pressure determinations on the 3,4,and 5% solutions of glycerol pectate neutralized and adjusted to 85% esterification are shown in Graph I. The 4% solution gave a final oncotic pressure comparable to human plasma and to the 6% dextran solution used as reference material. With respect to plasma the 3% level was hypotonic and the 5% level accordingly hypertonic.


Graph I
Oncotic Pressures of Glycerol Pectate Solutions
Measured in physiological saline
Esterification Degree: 85%
Oncotic Pressure in mm H2O
700
600
500
400
300
200
100
20
40
60
80
100
120
140
Time in HOURS
5% (η=4.3)
6% dextran
4% (η=4.4)
3% (η=3.3)

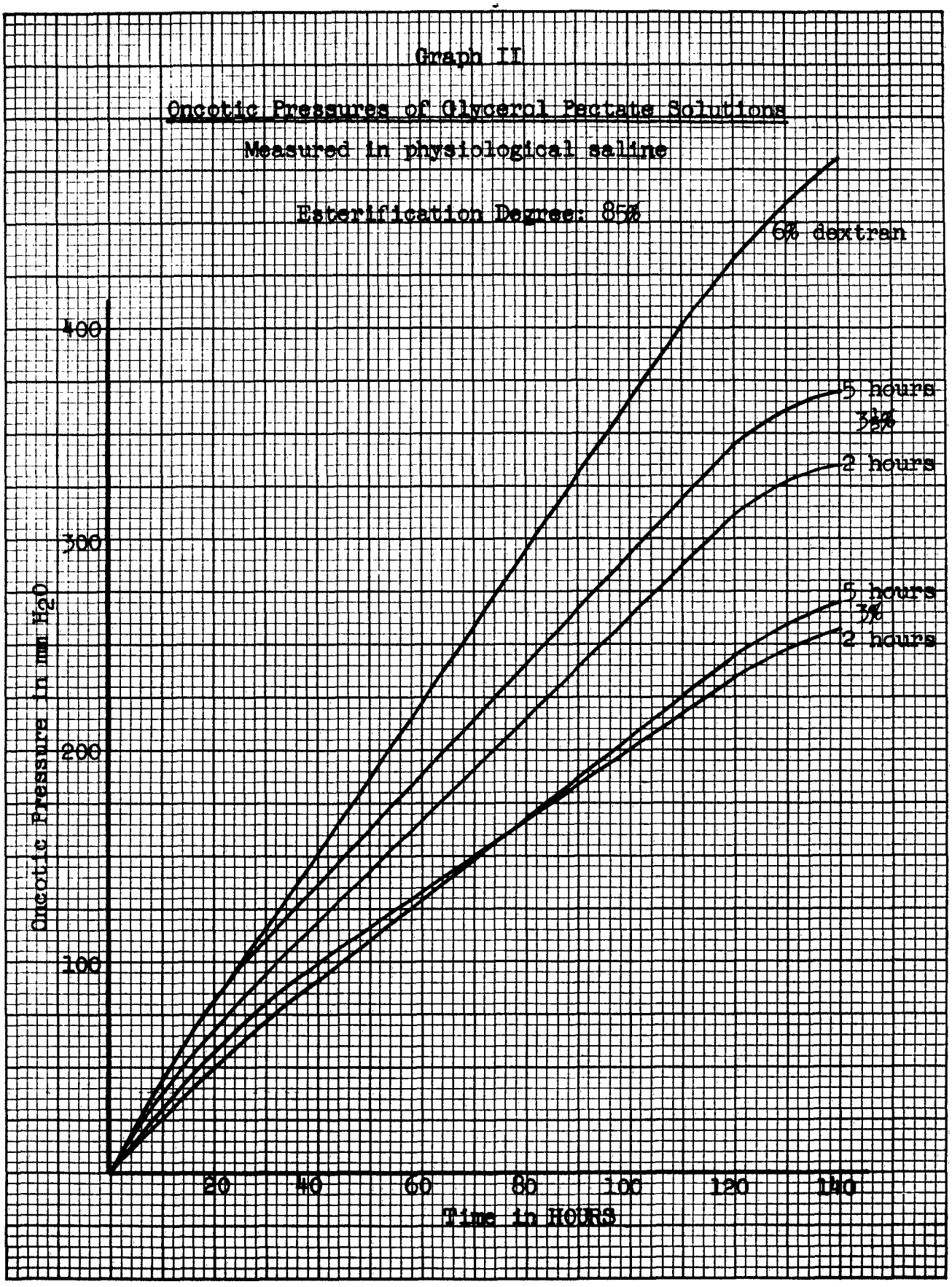
Further thermal degradation of the 4% solution to a relative viscosity of 3.3 effected a slight increase in oncotic pressure, as expected. It was thus of interest to find out whether solutions at levels slightly less than 4% may be prepared which upon extended thermal degradation may yield oncotic pressures equal to the 4% level. As shown in Graph II the oncotic pressure of 3% and 3½% solutions of glycerol pectate thermally degraded for 2 and 5 hours, respectively, were obviously much lower than that of plasma or 6% dextran solutions. Even at the 3½% level a very much longer degradation time would have been necessary to raise the pressure to that of 6% dextran solutions. This could have only been accomplished at a great sacrifice of molecular weight. It was therefore generally agreed to limit exploratory evaluation studies in animals to the 4% level in the relative viscosity ranges of 3 to 3½ and 4 to 4½. Depending upon the results obtained under these conditions, the effect of varying the degree of esterification was to be investigated later on.

Approximately 125 liters of 4% solution of glycerol pectate were prepared for testing at Camp Lejeune, and for drying and stability studies. One-third of these preparations were in the lower relative viscosity range of around 3.3 (Lot #12-3A, #12-3B, 13-3A and 13-3B), whereas the major part was at a relative viscosity of 4. Table II shows the individual batches made and illustrates the degree of uniformity obtainable by proper degradation and partial saponification.

TABLE II

4% Glycerol Pectate Lot #	Relative Viscosity	Unadjusted Free COOH meq/g	Ester %	Adjusted[1] Free COOH meq/g	Ester %	Oncotic Pressure[2] %
11	4.4	0.433	89.2	0.590	85.2	95
12-3(A)	3.3	--		0.573	85.6	103
12-3 B	3.2	0.496	87.6	0.598	85.0	--
12-4(A)	4.4	0.457	88.6	0.580	85.5	90
12-4 B	4.3	0.457	88.6	0.587	85.3	93
12-4 C	4.3	--		--		--
12-4 D	4.1	--		--		80
13-3 A	3.1	0.488	87.8	0.601	85.0	--
13-3 B	3.2	--		--		--
13-4 A	4.2	0.438	89.1	0.610	84.7	92
13-4 B	4.0	--		--		--
14-4 A	4.4	0.468	88.3	0.595	85.1	91
14-4 B	4.5	--		--		--
14-4 C	4.4	--		0.604	84.9	79

[1]Neutralized and partially saponified to 85% esterification with sodium hydroxide except for Lot Nos. 12-4D and 14-4C which were neutralized and adjusted with sodium bicarbonate.
[2]In comparison with 6% dextran


Graph II
Oncotic Pressures of Glycerol Pectate Solutions
Measured in physiological saline
Esterification Degree: 85%
6% dextran
5 hours
3½%
2 hours
5 hours
3%
2 hours
Oncotic Pressure in mm H2O
400
300
200
100
20
40
60
80
100
120
140
Time in HOURS

It should be emphasized that in preparing the glycerol pectate for these solutions, two different batches of Pectic Acid No. 75 were used, and that reaction conditions and isolation procedures were gradually modified to improve the process and yields. It, therefore, appears that differences in the purified glycerol pectate do not affect the properties of the final solution, provided the proper degradation time and partial saponification are carried out.

As already pointed out, glycerol pectate shows an optimum stability at a pH of about 4, the natural pH of pectin. It has therefore been necessary to store the degraded solutions at this pH and to neutralize them just prior to administration. As this was thought to be inconvenient, attempts were made to develop a neutral product of satisfactory stability. These studies led to a reasonably stable solution, and a dry material suitable for extended storage as described in the following:

The initial procedure of neutralizing the pasteurized and degraded solutions of glycerol pectate with sodium hydroxide just prior to use, caused considerable handling difficulties. When the glycerol pectate solution was not adequately agitated the alkali was used up locally before it had a chance to become evenly distributed. Substitutes for sodium hydroxide were then investigated among which sodium bicarbonate appeared most promising. When an equivalent amount of bicarbonate (equal to the amount of NaOH calculated for neutralization and partial saponification of excess ester to 85%) was added, the pH remained below neutrality. It could be shown that the slightly acid pH was due to dissolved carbon dioxide. The latter could be removed by aeration with nitrogen whereupon the pH increased to about 7.3 to 7.4. Solutions neutralized with NaOH showed an initial pH rise beyond neutrality followed by a gradual decline to a pH of 5 to 5.5 (after 2 weeks); sodium bicarbonate differed from NaOH in that it exerted an obvious buffering action.

As it seemed advantageous to have a colorless parenteral solution, a small amount of chlorine dioxide was added to the degraded solution of glycerol pectate before buffering with sodium bicarbonate. This measure was found of great importance as it prevented (presumably due to chemical sterilization) any visible contamination of the solutions. Since the chlorine dioxide completely disappeared in the first 48 hours, and since its decomposition products were hydrochloric acid and oxygen, it was regarded a safe means of destroying the last traces of proteinaceous matter and impurities other than polysaccharides. Table III shows the course of the pH of a 4% solution of glycerol pectate treated with ClO_2 and neutralized with bicarbonate. Since its preparation in November 1953, no visible contamination has been detected, and the buffering action was still effective after 70 days of storage at room temperature. Similar shelf-life tests, on solutions in sterile containers, have confirmed this result to date; they are being continued to determine the practical shelf-life of bicarbonate solutions. Sterility tests will also be made to establish the effectiveness of chemical sterilization with chlorine dioxide.

TABLE III

Time	pH Buffered with NaHCO3	pH CO2 Removed
1 Hour	6.5	7.4
2½ Days	6.0	7.4
3½ Days	6.0	---
10 Days	5.7	7.4
18 Days	5.4	6.4
38 Days	5.2	6.7
70 Days	5.2	6.6

Another approach to developing a stable product was seen in a dry product of glycerol pectate which would only require sterile water for reconstitution. Several methods of drying properly degraded 4% solution of glycerol pectate in normal saline have been investigated and found effective. Spray-drying, drum drying and lyophilization gave nonhygroscopic products with good to excellent dissolving properties. The dry materials, upon reconstitution with water, had substantially the same characteristics as their original solutions before drying. At present, lyophilization is the preferred drying method as all equipment required is readily available or can be easily assembled from standard laboratory equipment.

Discussion

The present status of the work on glycerol pectate allows some very interesting deductions and speculations with regard to its action as a plasma volume expander. The pectin solutions (sometimes referred to also as pectin sols) used by Hartman and others have consisted of a 1½% solution of degraded Pectin N.F. with a relative viscosity of around 4-5. They have shown a number average molecular weight of 18,000 (6) and a degree of esterification of about 60%. When we tried to prepare a suitable parenteral solution from highly esterified Pectin N.F., obtained by esterification of the free carboxyl groups with glycidol, we found we had to resort to higher concentration levels in order to attain oncotic pressures equal to plasma. It was quite evident that the loss of oncotic pressure on esterification was not due to the slightly higher average molecular weight and thus not due to a smaller number of molecules at a given concentration, but that it was in direct proportion to the decrease of free carboxyl groups. As pointed out before, the same was true for glycerol pectate where relatively minor variations in the degree of esterification brought forth significant changes in oncotic pressure in vitro as well as in response in vivo, whereas variations in molecular weight, as affected by various degrees of degradation, were without significant effect. As a consequence, the number of free carboxyl groups including the sodium ions associated therewith was a contributing factor

[6] T.H. Schultz et al. J. Am. Pharm. Assn. 41, 251 (1952)

for oncotic pressure. This is illustrated in Graph III where oncotic pressure is plotted against number of free carboxyl groups expressed in milliequivalents per liter. It happens from the curve shown that between 24 and 26 meq/liter of unesterified carboxyl groups are necessary to attain oncotic pressure equal to 6% dextra solution. This would indicate that there is a practically unlimited number of combinations of concentration and degree of esterification that will fulfill these requirements. So, for instance, do the earlier pectin sols at 60% esterification and at 1.5% level. The possible compositions of solutions of pectic substances having a constant total free carboxyl content of 24 meq/liter are represented by the calculated curve shown in Graph IV. Any combination of degree of esterification and concentration of pectic substances which falls on this curve should show equivalent oncotic pressure. However, the useable combination range is limited by two factors, (a) the threshold for calcium precipitation (at about 70% esterification) and (b) the maximum permissible relative viscosity of the solution (at about 5%). While the 4% level at 85% esterification was studied more extensively and found satisfactory in animals, other combinations falling on the curve of Graph IV, around the 4% level, seem feasible to produce adequate oncotic pressure and may be also satisfactory as a plasma replacer. Compared with other plasma expanders such as dextran or PVP, glycerol pectate has as an additional degree of freedom, the degree of esterification, which allows a broader concentration range, and thus, in turn, also a broader molecular weight range.

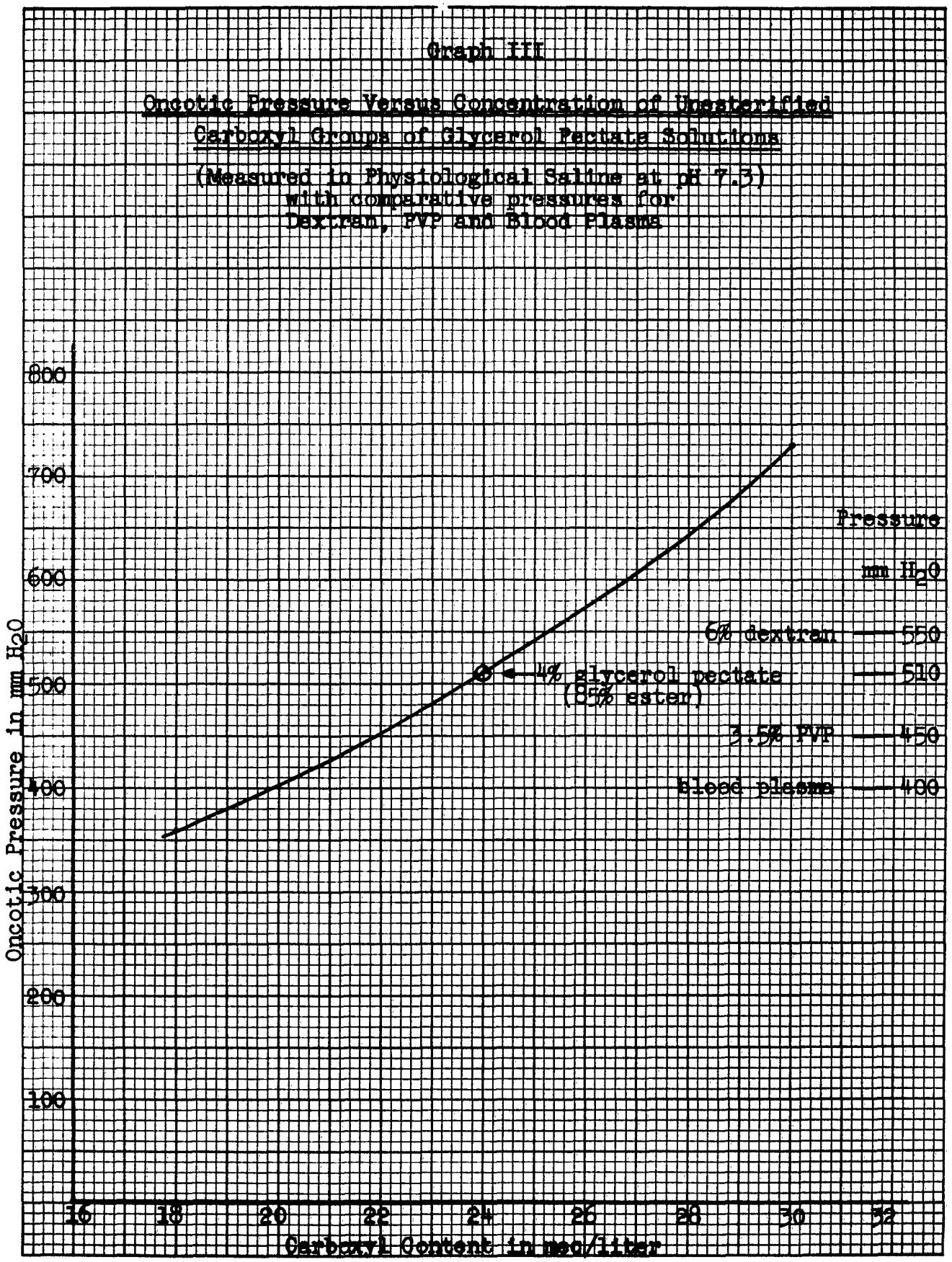
Arrangements have been made with the National Bureau of Standards for molecular weight determinations on glycerol pectate solutions. In lieu of these measurements predictions can be made of the approximate magnitude. Based upon the determination of 1.5% pectin sols of around 4 viscosity, it is conceivable that at 4% concentration and 4 viscosity, the average molecular weight will be about one-half of 18,000. Thus, assuming an average molecular weight of 10,000 for glycerol pectate, a molecule would be composed of 40 glycerol anhydrogalacturonate units. At 85% esterification there would be 6 free carboxyl groups per molecule surrounded by an equal number of sodium ions. Both the carboxyl and the sodium ions are likely to be hydrated. The arrangement of hydrated sodium ions around the polymeric ester acid may contribute to an apparent molecular size greater than expected from the true molecular weight, thus being retained in the circulatory system for a sufficient length of time to restore and maintain plasma volume in shock.

Our observations have shown that at a given concentration, viscosity and degree of esterification, the oncotic properties of glycerol pectate vary depending upon the amount of electrolyte present. For example, solutions in pure water have a 3-4 times greater oncotic pressure than in saline. In the circulatory system the oncotic pressure of glycerol pectate will also be influenced by the concentration of electrolytes and presumably also by the presence of plasma proteins. It is therefore conceivable that glycerol pectate is excreted more rapidly when administered to normal dogs since high electrolyte and plasma protein levels would be expected to depress the degree of hydration and thus reduce the apparent molecular configuration. In hemorrhaged dogs

Graph III

Oncotic Pressure Versus Concentration of Unesterified Carboxyl Groups of Glycerol Pectate Solutions

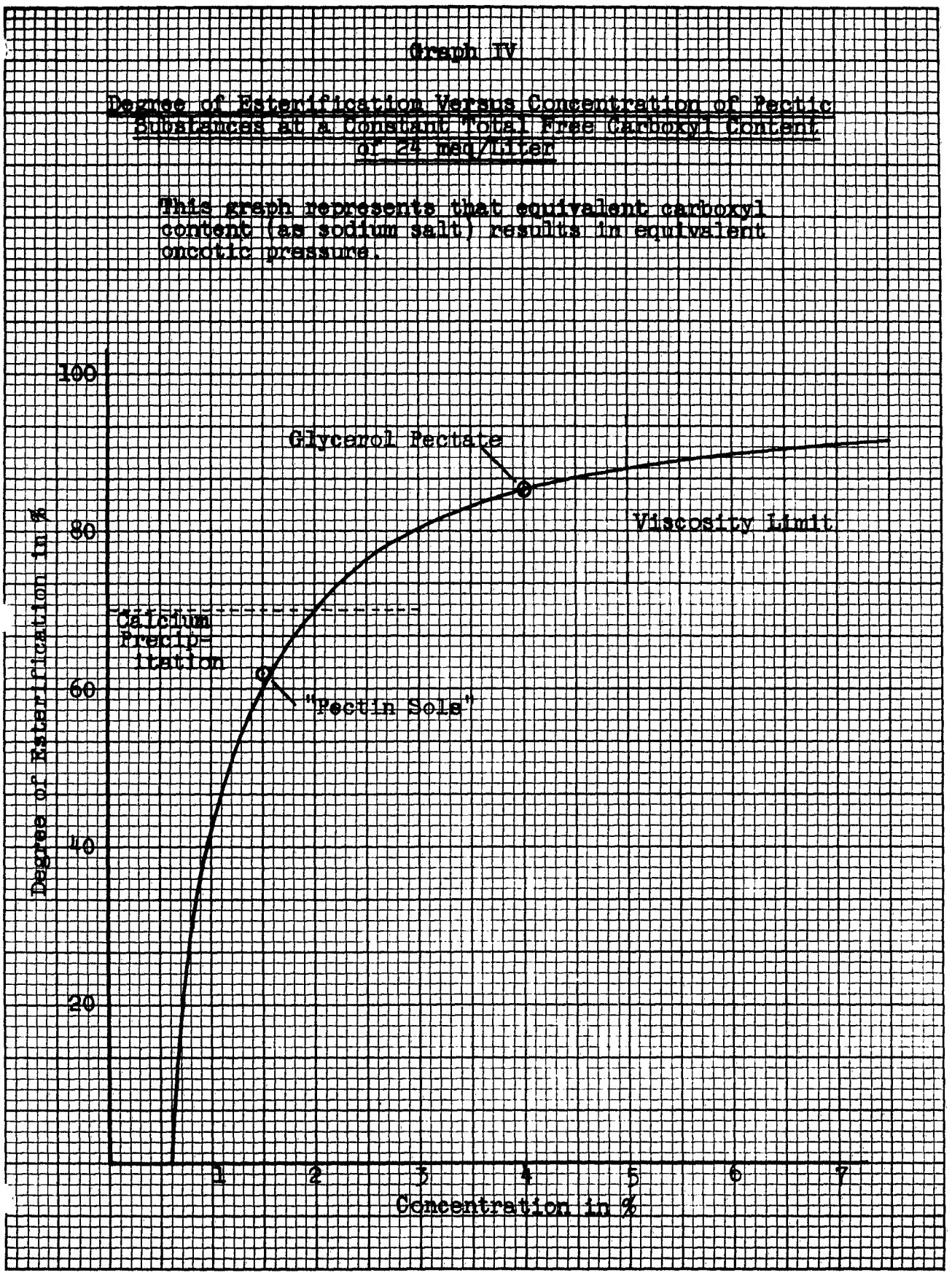
(Measured in Physiological Saline at pH 7.3) with comparative pressures for Dextran, PVP and Blood Plasma



Graph IV

Degree of Esterification Versus Concentration of Pectic Substances at a Constant Total Free Carboxyl Content of 24 meq/Liter

This graph represents that equivalent carboxyl content (as sodium salt) results in equivalent oncotic pressure.



the opposite effect should be observed, that is, retention should occur due to an increase in hydration until normal plasma protein and electrolyte levels are restored. The same phenomenon may also be explained by a charge effect on the membrane, in vitro as well as in vivo, which is also dependent upon the concentration of electrolytes.

Whatever the correct explanation may be, the fact that glycerol pectate, at an average molecular weight considerably below that of plasma protein, has proven to be an effective plasma replacer is of great significance. Once the plasma volume is restored to normal the glycerol pectate can leave through the kidney much more easily than a synthetic plasma expender with an average molecular weight exceeding that of plasma proteins, for example, dextran. Where emphasis is placed in avoiding deposition in the tissue an effective synthetic replacer with an average molecular weight below the barrier of the normal kidney is undoubtedly preferred.

Program for Future Research Studies

Future work and research studies are contemplated on:

1) Preparation of glycerol pectate for biological studies at Camp Lejeune and for molecular weight determinations at the National Bureau of Standards.
2) Further studies on the relationship between the oncotic pressure and the percentage of free carboxyl groups present in the glycerol pectate.
3) Continuation of studies on the stability of glycerol pectate solutions and on the development of a dry product suitable for extended storage.
4) Improvements on the synthesis of glycidol.
5) Esterification of pectic acid in a heterogenous system with lower quantities of glycidol.
6) Analytical methods for determination of glycerol pectate in blood, urine and possibly tissue.

Acknowledgment

This research was carried out under grants from the Office of Naval Research and the Office of the Surgeon General through the Office of Naval Research.

Appreciation is expressed for interest in this project to Colonel John R. Wood of the Office of the Surgeon General and to Drs. R. Robertson, O. E. Reynolds, Lewis Larrick, Paul Lindsay and Captain C. W. Schilling, and we are appreciative of guidance in this research shown by Commander Dr. W. J. Perry, Head, Clinical Branch of the Office of Naval Research.

Grateful acknowledgment is given to the staff of the Naval Medical Field Research Laboratories at Camp Lejeune for providing the biological evaluation of the product prepared under this research contract and especially to Captain Dr. C. B. Galloway, Commander Dr. S. W. Handford, Drs. C. M. Smythe, R. S. Leopold and R. H. Kathan for their continued guidance and help in this project. Dr. Handford has accomplished the biological evaluation of the materials supplied to him in a remarkably short period of time due to his undaunted efforts.

Early evaluation of the products of this research were undertaken by Dr. F. W. Hartman and Dr. Vivian C. Behrman at the Henry Ford Hospital, and it was this work that provided the biological background for later research studies. Dr. Hartman has been continually available for counsel and advise on this project for which we are greatly indebted.

Dr. Urs Nager has written this report covering this research work in which he was the principle investigator with Drs. E. E. Stahly and R. G. Jennen and the undersigned contributing a part of their time and acknowledgment is hereby made for their contribution.

The biological studies relating to glycerol pectate will be reviewed in a report by Dr. Handford.

Oliver W. Burke, Jr.